X-ray Structure Determination of GM2-activator Protein and its ligand Complexes

C. Wright, S. Li, and F. Rastinejad (U. of Virginia)

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Beamline(s): **X12C**

Introduction: The crystal structure of GM2-activator Protein (GM2AP) has been successfully determined by MAD using data collected at the X12C beamline during the period Nov. 19-21, 1998.

The structure turned out to be very interesting revealing a new fold and a deep cavity suitable for binding lipid moieties of ganglioside ligands. A publication describing the structure is presently in press (J.Mol. Biol.; Wright, Li and Rastinejad).

Data collection and structure determination: Crystals of the selenomethione modified protein, either unsubstituted or soaked in trimethyl lead acetate), were transported to NSLS in the frozen state. Data measurements were made on two crystals. One of these (a trimethyl lead acetate soak) was of better quality and was thus used to carefully measure anomalous data at five wavelengths. Two wavelengths were chosen at the lead edge (0.9485, 0.9470) and two at the selenium edge (0.9756, 0.9760) and one at a wavelength in-between the Pb and Se edges (0.9611). The resolution limit of the crystal was 2.3 -2.4 Å. The data were processed and scaled on-site using DENZO/ Scale pack and were of excellent quality yielding values for R-merge of 3.6 - 4.3%. The program SOLVE was used to locate the heavy atoms sites and determine phases. This was a lengthy process. It turned out that there was no lead signal. However, the two datasets collected at the Pb-edge were useful for phasing as 'remotes'. Difficulties were also encountered in obtaining consistent results for the positions of the selenium sites. With 2 SeMet/171 residues in the molecule and 3 monomers in the asymmetric unit, only 3 sites could finally be located with confidence. Neither of these was fully occupied and the resulting phases did not produce an interpretable electron density map without the aid of density modification (55% solvent level). The structure revealed that both methionines are exposed and partially disordered (high atomic B-factors), explaining our difficulties in using the selenium sites for phasing. Structure refinement was carried out with CNS against a 2.0 Å dataset collected earlier at CHESS.